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Regulation of *glnA* Messenger Ribonucleic Acid Synthesis in *Klebsiella aerogenes*

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We examined wild-type and mutant strains of *Klebsiella aerogenes* for the relative amounts of ribonucleic acid (RNA) hybridizing specifically to deoxyribonucleic acid from a transducing phage carrying $glnA_K$, the structural gene for glutamine synthetase. Our data showed a positive correlation between the intracellular level of glutamine synthetase and the level of *glnA* messenger RNA; we were unable to detect *glnA* messenger RNA in strains devoid of glutamine synthetase protein. Therefore, it is possible that transcription of *glnA* is not regulated simply by repression mediated through the glutamine synthetase protein; rather, autogenous control in this system may involve activation of transcription. Our experiments also suggest that the promotor of the *glnA* gene is located at the *rha* proximal end of the gene.

Glutamine synthetase (EC 6.3.1.2) catalyzes a reaction in which ammonia combines with glutamate to yield glutamine. This reaction is responsible for the biosynthesis of glutamine in a wide variety of organisms. Moreover, in enteric bacteria grown under conditions of nitrogen limitation, the reaction catalyzed by glutamine synthetase is coupled with that catalyzed by glutamate synthase (EC 2.6.1.15) to serve as the only means for incorporating free ammonia into cellular metabolism (9, 14). Thus, the enzymatic properties of glutamine synthetase are of critical importance to cells of enteric bacteria.

In addition, it appears that glutamine synthetase regulates transcription in these cells. This idea was originally proposed by Magasanik and co-workers from physiological studies on *Klebsiella aerogenes* (9, 11). It was then tested in a transcription system containing only highly purified components (16); the results clearly showed that the glutamine synthetase protein per se can specifically activate transcription by ribonucleic acid (RNA) polymerase of deoxyribonucleic acid (DNA) coding for enzymes that supply the cell with glutamate and ammonia. Recent experiments with cells of *K. aerogenes*, (9) *Klebsiella pneumoniae*, (12) and *Escherichia coli* (7) strongly indicate that glutamine synthetase regulates transcription of a variety of genes, all involved in nitrogen assimilation.

Therefore, it is of interest to ask what regulates glutamine synthetase activity in these cells. Previous studies with *E. coli* and *K. aerogenes* have demonstrated two levels of control: (i) the enzymatic (6, 17) and regulatory (9) properties of this protein are altered by the covalent attachment of adenosine 5'-monophosphate residues, and (ii) the intracellular level of the glutamine synthetase protein can vary (6, 9, 17). Both of these changes occur in response to the available supply of nitrogen. From studies with *K. aerogenes* on the genetics and biochemistry of the proteins affecting the reversible adenylylation reaction, Magasanik and coworkers have proposed (9) that the glutamine synthetase protein regulates its own synthesis at the level of transcription, thereby effecting the increase in nonadenylylated glutamine synthetase observed under conditions of nitrogen limitation.

This notion of autoregulation by glutamine synthetase is based on two sets of observations. First, mutations leading to a high constitutive level of glutamine synthetase protein appear to map within *glnA*, the structural gene for the glutamine synthetase polypeptide (4, 13). Second, lesions in the structural genes for proteins involved in the glutamine synthetase adenylylation system also alter the rate of production of glutamine synthetase in an inverse manner to their effects on the adenylylation state of the enzyme (5, 9).

The experiments reported in this paper were undertaken to gain additional information about regulation of glutamine synthetase in *K. aerogenes*. We have used the technique of RNA-DNA hybridization to measure the rela-

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tive amounts of $glnA_K$ messenger RNA (mRNA) in cells of *K. aerogenes*. Our data are most easily explained by a model in which regulation of synthesis of glutamine synthetase occurs at the level of transcription and is affected by the conformational state of the glutamine synthetase protein. Our results appear to establish the direction of *glnA* transcription on the bacterial chromosome.

MATERIALS AND METHODS

Bacterial strains. *K. aerogenes* strains employed were obtained from the collection of B. Magasanik. They are listed in Table 1, together with *E. coli* strains used for growing and titering of ϕ80 and ϕ80*glnA* phage.

Phage. The ϕ80$glnA_K$ transducing phage was isolated (Tyler, manuscript in preparation) from a lysate of phage ϕ80d*metBJFrha* obtained from a hybrid strain of *E. coli* carrying the $glnA_K$ gene (15). The ϕ80d*metBJFrha* phage was derived from the ϕ80d*met(K)* phage (10) by B. Conrad. The ϕ80 helper phage was ϕ80h, obtained from L. Soll.

Media. The composition of the complex medium (LB) and minimal medium (W) have been described previously (15). Minimal medium was supplemented at final concentrations of: glucose, 0.4%; histidine, 0.4%; $(NH_4)_2SO_4$, 0.2%; glutamine, 0.2%.

Assay of glutamine synthetase enzyme activity. Glutamine synthetase was assayed in whole cells as described by Prival et al. (11).

Production and purification of phage. Wild-type ϕ80 phage was obtained by lytic infection of the strain EG47 grown in LB medium. Phage were concentrated by polyethylene glycol precipitation and purified through cesium chloride block and equilibrium gradients as described previously (16).

To obtain the ϕ80*glnA* phage, strain T258 was grown at 30°C in a 20-liter New Brunswick fermenter to a density of about 80 to 100 Klett units. The prophage was induced with mitomycin C (2 μg/ml), and 0.5 h later ϕ80 helper phage was added. The cells were grown until lysis occurred (usually 4 to 5 h), chloroform was added, and the lysate was passed through a centrifuge (Sharples) to remove debris. The supernatant contained 10^8 to 10^9 plaque-forming units per ml (helper phage) and 10^6 to 10^7 *glnA* transducing particles (ϕ80$glnA_K$ phage) per ml. The phage were concentrated with polyethylene glycol and banded in cesium chloride block gradients. Then ϕ80 and ϕ80*glnA* phage were separated by centrifugation for 60 h at 35,000 rpm in a centrifuge (International; rotor A321) in a cesium chloride equilibrium gradient. The upper band, containing the majority of the transducing particles, was collected and purified once more in an equilibrium gradient. The final phage stock solution contained 10^{10} to 10^{11} *glnA* transducing particles per ml. After cesium chloride banding phage were dialyzed for at least 48 h against 10^{-2} M tris(hydroxymethyl)-aminomethane (pH 7.4) – 10^{-2} M $MgSO_4$ buffer containing decreasing amounts (from 1.0 to 0.1 M) of NaCl.

Extraction of DNA. Phage DNA was phenol extracted as described by Cooper et al. (3).

Preparation of single DNA strands. Phage DNA strands were separated according to the method of Hradecna and Szybalski (8).

Labeling and extracting of RNA. Cells were grown in W medium up to 100 Klett units at 30°C in fast-shaking Erlenmeyer flasks, and 2.5-ml cultures were labeled with [^{3}H]uridine (500 μCi; 5 μg/ml) for 8 min. Preliminary experiments were performed to ensure that uptake of uridine is linear for this period of time with these conditions. Before phenol extraction of RNA, cells were harvested, made into spheroplasts, and lysed with sodium dodecyl sulfate buffer, as described by Cooper et al. (3).

Hybridization procedures. RNA was incubated with single-stranded phage DNA immobilized on nitrocellulose filters (Schleicher & Schuell, B6) for 24 h at 66°C after the procedure as described by Cooper et al. (3).

Hybridization of RNA with single-stranded DNA was carried out in liquid as described previously (15). Phenol-extracted RNA used in these experiments was additionally purified before hybridization by passing it through a G-50 Sephadex column (20 by 1 cm), followed by filtration through a (Millipore Corp., 0.45-μm pore size) nitrocellulose filter. Filters used in both hybridization techniques were dried, immersed in toluene scintillation fluid, and counted in a scintillation counter (Beckmann LS330).

Reagents. [5-H^3]uridine (specific activity, 26.2

TABLE 1. *List of strains*

Strain	Relevant genotype	Source
Klebsiella aerogenes		
MK9000	*glnA*$^+$	13
MK9011	*glnA6*	13
MK9021	*glnA10*	13
MK9028	*glnA4*	13
MK9042	*glnB3*	13
MK9052	*glnA5*	13
Escherichia coli		
EG47	*glnA*$^+$	15
T245	*glnA201* (ϕ80h)	This laboratory
T258	*glnA201* (ϕ80$glnA_K$)	This laboratory

Ci/mmol) was obtained from New England Nuclear Corp. All other chemicals were reagent grade and commercially available.

RESULTS

Standardization of the hybridization reaction with ϕ80*glnA* DNA. Several preliminary experiments were performed to establish the best conditions for detecting *glnA*-specific mRNA by means of RNA-DNA hybridization on filters. Since our yields of the ϕ80*glnA* phage were low, we examined whether we could load filters with small amounts of DNA and still get meaningful results. A number of RNA saturation curves were generated using sets of small filters (6 mm in diameter) loaded with various constant amounts of DNA and [^{3}H]RNA (from nitrogen-limited cultures of wild-type *K. aerogenes*) prepared as described in Materials and Methods. Several different times of hybridization were used for each of these early experiments. We found (Fig. 1) that when these small filters contain approximately 0.3 μg of ϕ80*glnA* DNA and the hybridization reactions are incubated at 66°C for 24 h, there is a linear increase in the counts per minute hybridizing to ϕ80*glnA* DNA as the input of RNA is increased up to 3 μg per reaction, at which point about 10,000 cpm are bound to the filter. The presence of a linear dose-response curve indicates that, for each concentration of input RNA, all hybridizable RNA is, in fact, complexing to the DNA under these reaction conditions (3). Since, in a control experiment, very little radioactivity (less than 500 cpm) was bound to similar filters loaded with ϕ80 DNA for any quantity of input RNA, we conclude that these conditions give a specific and reliable assay for the cellular RNA homologous to the bacterial DNA carried by this transducing phage. All subsequent experiments were performed with the assay illustrated in Fig. 1; and, because of the low quantity of DNA used in these reactions, we have always confirmed the linearity of our assay for bacterial RNA by running a series of reactions with different quantities of input RNA exactly as shown in Fig. 1.

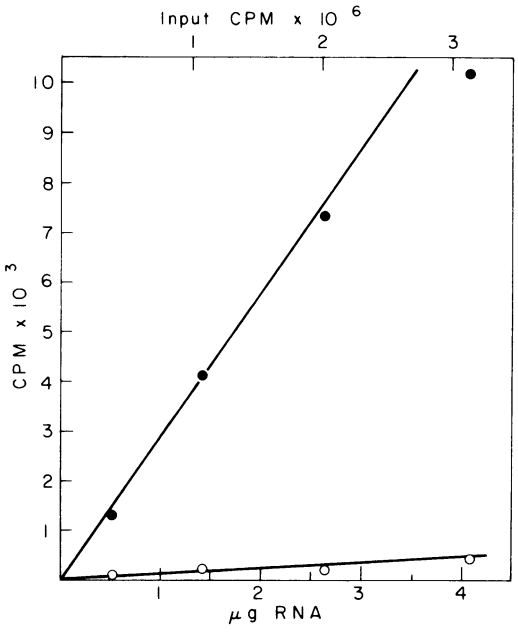


FIG. 1. *Hybridization of K. aerogenes RNA to DNA from ϕ80glnA$_K$ and ϕ80. [^{3}H]RNA was obtained as described in Materials and Methods from K. aerogenes MK9000 growing at 30°C in minimal salts medium (W) supplemented with glucose and glutamine. The specific activity of the RNA was determined by measuring the amount of trichloroacetic acid-precipitable in counts per minute and the absorbance at 260 nm of a sample of the preparation. Hybridization was carried out at 66°C for 24 h in a volume of 50 μl of RNA in 4 × SSC (1 × SSC = 0.15 M NaCl plus 0.015 M sodium citrate) (3) containing nitrocellulose filters loaded with approximately 0.3 μg of DNA. Symbols: ●, ϕ80glnA$_K$ DNA; ○, ϕ80 DNA.*

Determination of *glnA*-specific RNA in wild-type cells of *K. aerogenes*. The intracellular level of the glutamine synthetase protein can be determined either by measuring enzymatic activity directly or by complexing protein to antiserum prepared against purified glutamine synthetase; both assays agree quantitatively (R. A. Bender, Ph.D. thesis, Massachusetts Institute of Technology, Cambridge, 1976). Using these assays, one observed that the amount of the glutamine synthetase protein in the cell is dramatically affected by the supply of ammonia, glutamine, and α-ketoglutarate available to the cell. In particular, one finds that wild-type cells of *K. aerogenes* grown with glucose and excess ammonia contain about 10-fold less glutamine synthetase protein than cells grown in media containing glucose and a growth-rate-limiting source of nitrogen. Moreover, when these cells are grown in the presence of excess ammonia with high concentrations of glutamine and histidine as the source of carbon, the level of glutamine synthetase protein decreases still further, below the sensitivity of these assays (R. A. Bender, Ph.D. thesis, 1976).

To approach the question of autoregulation by glutamine synthetase, one needs to know whether these variations in the intracellular level of the glutamine synthetase protein correlate with changes in the intracellular concen-

tration of *glnA* mRNA. Therefore, we extracted RNA from cells of *K. aerogenes MK9000* grown under the conditions described above and hybridized this RNA to $\phi 80 glnA_K$ DNA and $\phi 80$ DNA. The results, given in Table 2, show that the variations in [^{3}H]RNA hybridizing specifically to DNA from the $glnA_K$ transducing phage correlate qualitatively, but not quantitatively, with the fluctuations usually observed for glutamine synthetase. However, since we do not know whether the bacterial DNA carried by the $\phi 80 glnA_K$ phage is confined only to the $glnA_K$ gene, we cannot assume that the RNA hybridizing specifically to DNA from this transducing phage represents only $glnA_K$ RNA; rather, we must deduce what proportion of this RNA is specific for the $glnA_K$ gene.

It seems unlikely, though possible, that unknown genes adjacent to *glnA* are also derepressed by nitrogen limitation. From this assumption, it follows that all $\phi 80 glnA_K$-specific RNA found in glucose-grown cells under conditions of nitrogen limitation (Table 2, line 1, Ggln medium), but not under conditions of excess ammonia (Table 2, line 2, GNgln medium), represents $glnA_K$ mRNA. Moreover, we found that there is a small, but very reproducible, decrease in the [^{3}H]RNA bound specifically to $\phi 80 glnA_K$ DNA when we use RNA from cells grown with excess ammonia but histidine and glutamine (HNgln medium), rather than glucose, as a source of carbon (compare $\phi 80 glnA_K$-specific RNA in lines 2 and 3 of Table 2). This decrease correlates with our assays for glutamine synthetase; this protein is found in cells grown in GNgln medium but is not detected in cells grown on HNgln medium. Consequently, we considered the possibility that RNA coding for the glutamine synthetase protein is found in cells grown in GNgln medium (Table 2, line 2) but is not present in cells grown on HNgln medium (Table 2, line 3). Applying this analysis, we obtained, by subtraction, values for the intracellular levels of translatable $glnA_K$ RNA which agree very well, quantitatively, with the 10-fold derepression usually observed for glutamine synthetase in cells grown under conditions of nitrogen limitation.

Nevertheless, the possibility still remained that our assay for enzymatic activity of glutamine synthetase is less sensitive than the hybridization assay, and that cells grown on HNgln medium do contain some $glnA_K$ mRNA, which is translated into small amounts of protein undetectable by our assays for this protein. However, the data plotted in the graph of Fig. 2 argue against this idea. Here one sees that a linear relationship exists between the amount of glutamine synthetase detectable in various cultures of *K. aerogenes* and the percentage of labeled RNA in these cells which hybridizes specifically to $\phi 80 glnA_K$ DNA. Extrapolation of the curve indicates that even when no glutamine synthetase is present in the cell, 0.05% of the labeled RNA is specific for the $\phi 80 glnA_K$ transducing phage. In other words, 0.05% of the $\phi 80 glnA_K$-specific RNA detectable under our experimental conditions is not translated into glutamine synthetase by these cells. Thus, these data extend the analysis presented above attempting to quantitate an assay for translatable *glnA* RNA.

Measurement of $glnA_K$ RNA in mutants altered in the production of glutamine synthetase. Using this assay, we estimated the amount of translatable *glnA* RNA in a variety of strains with different mutations in *glnA* or *glnB*, the structural gene for PII, one of the proteins of the adenylylation system. The data (Table 3) show that, with one exception (*glnA10*), the RNA detectable by our hybridization assay in these strains correlates well with the enzymatic activity of the intracellular glutamine synthetase.

Hybridization with the separated strands of DNA from the $\phi 80 glnA_K$ phage. It is of interest

TABLE 2. *Assay of $glnA_K$ mRNA in K. aerogenes*[a]

Growth medium	[^{3}H]RNA hybridized to:		$\phi 80 glnA_K$-specific RNA		$glnA_K$ RNA
	$\phi 80 glnA_K$ DNA (cpm)	$\phi 80$ DNA (cpm)	cpm	% of [^{3}H]RNA	% of [^{3}H]RNA
Ggln	6,850	190	6,660	0.33	0.28
GNgln	1,760	220	1,540	0.08	0.03
HNgln	1,260	200	1,060	0.05	0

[a] Cells of wild-type *K. aerogenes* MK9000 were grown at 30°C in minimal salts medium (W) with various supplements. Ggln medium containing glucose and glutamine results in a condition of nitrogen limitation. GNgln medium supplemented with glucose, glutamine and ammonium sulfate, and HNgln medium supplemented with histidine, glutamine, and ammonium sulfate, are conditions of nitrogen excess. [^{3}H]RNA was obtained and hybridized with DNA as described in Materials and Methods and illustrated in Fig. 1. The values indicated for counts per minute hybridized were obtained with 2×10^6 cpm of input [^{3}H]RNA.

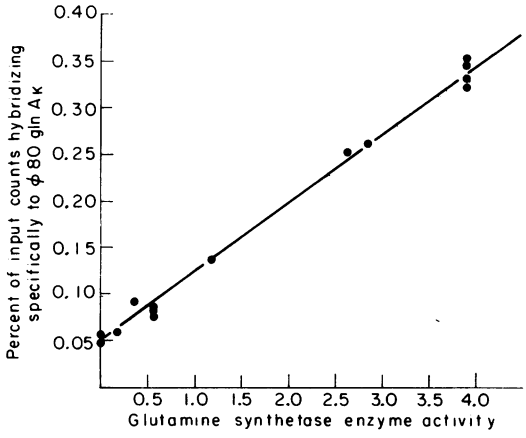


FIG. 2. *Relationship between $\phi 80 gln A_K$-specific RNA and glutamine synthetase enzyme activity. Cultures of wild and mutant strains of K. aerogenes were grown in minimal salts medium (W) containing various supplements. A portion of the culture was assayed for glutamine synthetase enzyme activity; [^{3}H]RNA was obtained from the remainder of the culture as described in Materials and Methods and hybridized with $\phi 80 gln A_K$ DNA and $\phi 80$DNA using the conditions and procedure described in Fig. 1. Each point represents the data from a different culture. Enzyme activity is expressed as units per milliliter of culture.*

TABLE 3. *Level of $gln A_K$ mRNA in strains of K. aerogenes*[a]

Strain	Genotype	Growth medium	Glutamine synthetase activity (units/ml)	$gln A_K$ RNA (% of [^{3}H]RNA)
MK9000	*glnA*$^+$	GglN	3.9	0.28
		GNgln	0.5	0.03
		HNgln	<0.01	0.0
MK9028	*glnA4*	Ggln	2.8	0.21
		GNgln	2.6	0.20
MK9011	*glnA6*	Ggln	<0.01	0.0
		GNgln	<0.01	0.0
MK9021	*glnA10*	Ggln	<0.01	0.22
		GNgln	<0.01	0.13
MK9052	*glnA5*	Ggln	0.2	0.01
		GNgln	<0.01	0.0
MK9042	*glnB3*	Ggln	1.2	0.08
		GNgln	0.2	0.01

[a] Growth conditions are those described in footnote *a*, Table 2. [^{3}H]RNA was obtained and hybridized with DNA as described in Materials and Methods and illustrated in Fig. 1. The percentage of [^{3}H]RNA corresponding to $gln A_K$ mRNA was calculated as described in the text.

to determine the direction of synthesis of this *glnA* mRNA on the bacterial chromosome. This information can be obtained by determining the direction of transcription of the $gln A_K$ gene on the transducing phage, if one makes certain assumptions about the molecular events that lead to formation of the $\phi 80 gln A_K$ phage. Therefore, RNA from the wild-type strain of *K. aerogenes*, MK9000, grown under conditions of nitrogen limitation, was hybridized to the separated strands of DNA extracted from phage $\phi 80 gln A_K$ and phage $\phi 80$. The data in Fig. 3 clearly show that only the heavy (R) strand of the DNA from the $\phi 80 gln A_K$ phage binds significant amounts of RNA. This result establishes the direction of transcription of the $gln A_K$ gene on the transducing phage, as illustrated in Fig. 4.

DISCUSSION

The experiments described in this paper investigate transcription of the $gln A_K$ gene, the

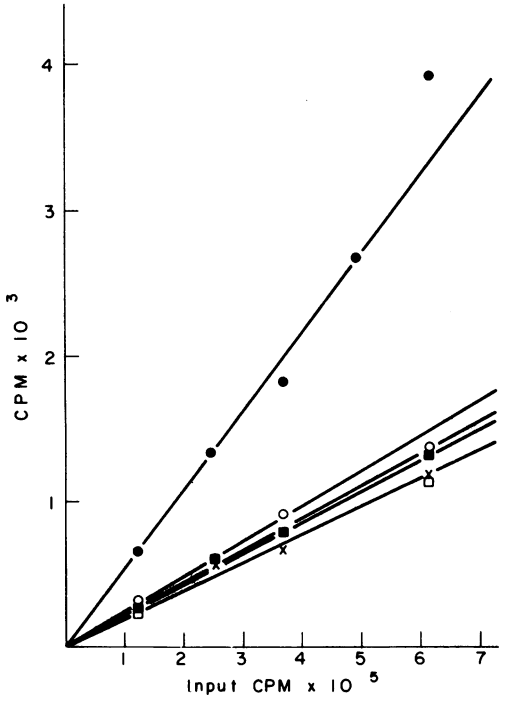
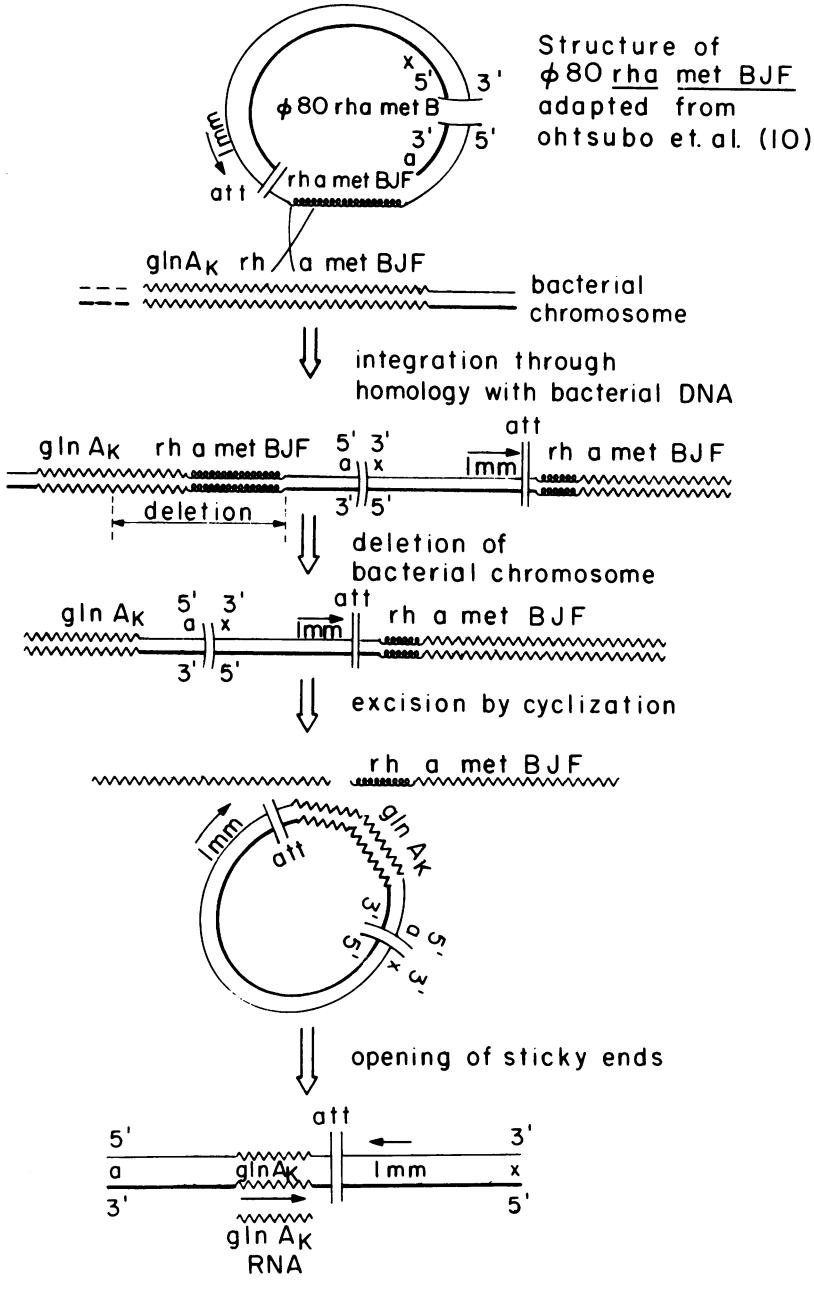


FIG. 3. *Hybridization of K. aerogenes RNA to the separated strands of DNA from $\phi 80 gln A_K$ and $\phi 80$. Cells of strain MK9000 growing on Ggln medium were used to prepare [^{3}H]RNA as described in Materials and Methods using the modification outlined under the section on liquid hybridization. The [^{3}H]RNA was hybridized in liquid to the single strands of DNA as described in Materials and Methods using various inputs of [^{3}H]RNA and a constant amount (0.6 μg) of DNA. Symbols: ●, H (heavy) strand of $\phi 80 gln A_K$ DNA; ○, L (light) strand of $\phi 80 gln A_K$ DNA; ■, H (heavy) strand of $\phi 80$DNA; □, L (light) strand of $\phi 80$DNA; ×, no DNA present.*



glnA$_K$ RNA hybridizes only to heavy strand of phage DNA in contrast to conventional observations for the immunity region

FIG. 4. *Model for the formation of φ80glnA$_K$ from φ80drha metBJF. φ80 sequences are shown as a straight line, bacterial sequences in the phage are shown by a circular line, and bacterial chromosomal sequences are indicated by a sawtooth line. The heavy straight line indicates the DNA strand conventionally observed to band at greater density in cesium chloride equilibrium gradients. att represents the normal phage attachment site, imm denotes the immunity region, and the arrow represents the direction of transcription. The sticky-end joint is denoted by the letters a and x with the numbers 3' and 5', indicating the polarity of the strands of DNA.*

structural gene for glutamine synthetase in *K. aerogenes* (4). In general, we find that the level of glutamine synthetase protein correlates directly with the level of *glnA* mRNA, as predicted from a model in which the synthesis of glutamine synthetase is regulated at the level of transcription. Our results indicate the direction of this transcription on the bacterial chromosome. In addition, this work shows that alterations in the structure of the glutamine synthetase protein affect the level of *glnA* transcripts in the cell.

We have assigned a direction to transcription of the $glnA_K$ gene on the $\phi 80 glnA_K$ phage by hybridizing RNA from cells of *K. aerogenes* to the separated strands of DNA from this transducing phage. The results demonstrate that *glnA* RNA is complementary to the heavy (or R) strand of $\phi 80 glnA_K$ DNA. Knowing this information, the orientation of the bacterial genes on the original $\phi 80$*rha met(K)* phage (10) that was used in isolating $\phi 80 glnA_K$ phage, and the relative chromosomal map position of the *glnA*, *rha*, and *metBJF* genes (1), we can assign a direction to transcription of the $glnA_K$ gene by making certain assumptions. We assume that the parental $\phi 80$*rha met(K)* phage integrated into the bacterial chromosome near $glnA_K$ by recombination with *metBJF* or *rha* and that the $\phi 80 glnA_K$ phage was formed by the simplest mistake in excision, which maintains the normal orientation of the chromosomal genes to one another. We also assume that the presence of bacterial DNA does not alter the relative positions of the L(l) and H(R) strands of phage DNA on cesium chloride equilibrium gradients. As illustrated in Fig. 4, by making these assumptions, and knowing that $glnA_K$ is transcribed from the heavy strand of DNA in this phage, we can argue that, on the bacterial chromosome, transcription originates at the end of the *glnA* gene proximal to the *rha* locus; that is, synthesis of *glnA* mRNA proceeds from the *rha* side of *glnA* toward *chlB*.

Our estimates of the intracellular $glnA_K$ mRNA in cells of *K. aerogenes* correlate well with the amount of glutamine synthetase detectable in wild-type strains and in strains carrying the *glnA4* or *glnB3* mutations. This result was expected for cells with the *glnA4* mutation, since this lesion results in constitutive production of high levels of glutamine synthetase. However, previous work has shown only that *glnB3* mutants have a low level of glutamine synthetase (5, 9, 11) when assayed by measuring enzymatic activity or by precipitation with antiserum prepared against nonadenylylated glutamine synthetase purified from wild-type cells. Since the altered P_{II} protein in these strains results in the abnormal production of highly adenylylated glutamine synthetase, the possibility remained that these assays did not accurately determine the amount of glutamine synthetase protein produced by such mutants. In this case, GlnB strains should contain a higher level of *glnA* mRNA than do wild-type cells with comparable amounts of glutamine synthetase enzyme activity. However, we find in *glnB3* mutants a very low level of *glnA* transcripts, corresponding exactly to that expected in wild-type cells with equal enzyme activity. Therefore, assuming that the stability of *glnA* mRNA is not affected by the altered P_{II} protein, our observations directly confirm the suggestion that adenylylation of glutamine synthetase decreases transcription of the *glnA* gene.

Our measurements of *glnA* RNA in strains with *glnA* lesions (*glnA10*, *glnA6*, *glnA5*), which eliminate essentially all detectable glutamine synthetase, are also relevant to theories on regulation on *glnA* transcription. We find that *glnA10* mutants have a high level of *glnA* RNA on both Ggln and GNgln media; however, we are unable to detect more than background amounts of $\phi 80 glnA_K$-specific RNA in strains carrying the *glnA6* or *glnA5* mutations, regardless of the media used for cell growth. These rather conflicting observations can be resolved to some extent by examining what is known of the physiology and genetics of strains carrying these different *glnA* mutations.

Strains with the *glnA10* mutation have the Cn^r phenotype; such cells always produce the Hut enzymes at a high rate in the presence of glucose. Therefore, it has been suggested that such a mutant always produces a *glnA* gene product altered in such a way that it can activate transcription of the *hut* operons, but it can not be detected by enzymatic assay or by antigen-antibody reaction (9). Our results are consistent with this proposal, which demands that strains carrying the *glnA10* mutation always contain high levels of *glnA* mRNA.

On the other hand, strains with the *glnA5* mutation apparently regulate the synthesis of glutamine synthetase but produce such extremely low levels of this protein that it is detectable, by assay for enzymatic activity or for glutamine synthetase antigen, only under conditions of nitrogen limitation (9). There is no evidence, by either assay, that *glnA6* strains produce any *glnA*-directed polypeptide capable of forming the dodecamer (5) that is active glutamine synthetase. In fact, genetic experiments indicate that *glnA6* may be a small deletion (13). It is possible that we are not recovering the *glnA* mRNA in these mutants because it is

extremely unstable due to lack of translation. However, there is precedent for recovering at least low levels of mRNA corresponding to genes carrying nonsense mutations that terminate translation (2). In fact, from intragenic mapping of *glnA* mutations (13) and from the direction of transcription which we propose for the *glnA* gene, the *glnA6* mutation may well be far distal to the operator-promotor region of this gene. In this case, one might expect that in strains carrying the *glnA6* mutation at least a portion of the *glnA* mRNA should, as a result of translation, be protected from degradation and consequently detected by our hybridization assay. Thus, our data may imply that mutations that greatly alter the primary sequence of the glutamine synthetase subunit also effectively eliminate formation of translatable *glnA* RNA.

We cannot, at present, exclude the possibility that the *glnA5* mutation results in a glutamine synthetase protein that functions as a superrepressor. However, we have no reason to believe that *glnA6* strains produce a complete glutamine synthetase polypeptide. Therefore, it may well be that the situation in the *glnA6* mutant does not conform to any proposal for autoregulation of glutamine synthetase which envisions only simple negative control. Such a model predicts that when the dodecamer of glutamine synthetase subunits is absent from the cell, the *glnA* gene will be transcribed at a high rate at all times. Hence, our observations are consistent with the idea that regulation of *glnA* transcription is not affected only by repression; rather, the conformation of glutamine synthetase may also affect activation of transcription of the *glnA* gene.

It is of interest to consider the nature of the $\phi 80 glnA_K$-specific RNA present in cells devoid of glutamine synthetase protein (Table 2 and Fig. 2). It is possible that this RNA is hybridizing to bacterial genes other than $glnA_K$ which are present on this transducing phage. However, it is also possible that this RNA represents a leader sequence on the $glnA_K$ gene that is always transcribed at high frequency but not translated.

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